Genomic compatibility excels possible 'good genes' effects of sexual selection in lake char

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Author contributions

LG, DN, and CW designed the experiment. LG, DN, and EL organized the field work. LG performed the 1st experiment, DN the 2nd, and CdG did the genomic analyses and calculated inbreeding coefficients and kinships. LG and LA monitored the embryos and determined growth. SJ provided the bacteria and prepared the infections. LG, DN, and CW did the statistics. CW wrote the manuscript that was revised by all authors.

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Abstract

Mating is rarely random in nature, but the effects of mate selection on offspring performance are still poorly understood, even in well-established models such as salmonid fish. We sampled wild lake char (*Salvelinus umbla*) and used their gametes to investigate the genetic consequences of different mating scenarios. In a first study, we used full-factorial breeding to experimentally separate additive genetic from maternal environmental effects. This led to 60 families and in total 1,073 embryos that were raised singly after sublethal exposures to the pathogen *Aeromonas salmonicida*, the common pollutant ethinylestradiol, or water only. Contrary to predictions of 'good genes' sexual selection, offspring of more yellow males were less tolerant to the pathogen than offspring of pale males, while male coloration did not predict offspring tolerance to ethinylestradiol. However, increased kinship between the parents had strong negative effects on embryo development in all treatment groups. In a second experiment, we monitored 1,464 singly-raised embryos that resulted from 70 pair-wise sperm competition trials. These embryos were raised in an environment that supports the growth of symbiotic microbes (sublethal stress) or in a clean environment. Offspring of yellow males were again less stress tolerant than those of pale males, and embryo development was again slowed down with increasing genetic similarity between the parents. We conclude that genetic benefits of mate selection would be strongest if females avoided genetic similarity during mate selection, for example based on MHC-linked signals, while male breeding colors seem more relevant in intra-sexual selection.

Introduction

Evolution is driven by natural and sexual selection, and the latter by intra-sexual competition and mate selection. Mate selection can go both ways, but female choice largely characterizes sexual selection in many species and can, in principle, happen before release of gametes (mate choice), after release of games but before fertilization (cryptic female choice), or after fertilization (differential investment). When selecting mates, females can get direct benefits (nuptial gifts, protection, paternal care, etc.) or indirect (genetic) ones that increase offspring fitness. The genetic consequences of mate selection are therefore best studied in species with external fertilization and little or no parental care, and ideally where female differential investment cannot be a potentially confounding factor. Salmonid fish are excellent models in this context because they spawn externally and leave their embryo to develop in the interstices of gravel. Moreover, much is known about their ecology and life history [1], while

full-factorial *in vitro* fertilization and the rearing of large numbers of embryos, each in its own container, allow separating the variance component (additive genetic effects and maternal environmental effects) and testing for various types of interactions with the necessary numbers of independent replicates.

Salmonid fish have polygamous spawning systems that have been discussed as potentially influenced by intra-sexual dominance, endurance rivalry, scramble competition, sperm competition, and mate choice [2,3]. Intra-sexual dominance is usually determined in fights or displays of fighting ability. Normally, larger males with well-developed secondary sexual characters win such dominance contests in salmonids [e.g. 4,5] and other animals [6]. Male reproductive success is also dependent on how long they can remain reproductively active during the breeding season (endurance rivalry), how close they can position themselves to the vent of spawning females on their own and on other males' spawning territories (scramble competition), and on their sperm number, motility, velocity, and longevity during simultaneous spawning with competitors (sperm competition). In comparison to these first four characteristics of spawning, female choice is often assumed to play a minor role in salmonid fish. However, females have often been observed to delay spawning when courted by non-desired males [7,8] or show aggressive behavior towards some types of males [9], i.e. female choice seems still possible [2,3].

Potential genetic benefits of mate selection can be grouped in two categories: additive genetic effects ('good genes') and non-additive genetic effects ('compatible genes') [10]. Additive genetic effects are typically assumed to be revealed by a male's health and vigor, because only males in good health and vigor can afford the costs that are linked to extra-ordinary colors, morphological structures, or behaviors that would potentially make males sexually attractive [6]. Additive genetic benefits of mate selection typically explain only a small fraction of the variance in fitness in various taxa [11,12]. In fish, additive genetic benefits can be strong [e.g. 13,14,15] or weak [e.g. 16,17] and have not been sufficiently understood yet. In general, indicators of 'good genes', i.e. of male health and vigor) are predicted to be conspicuous behaviors, morphology, and/or coloration [6]. Their information content could theoretically be high [18] but is expected to vary, for example, depending on male age in iteroparous species [19].

Mate selection for non-additive genetic effects may not be based on the costs of conspicuous male traits [20] but is instead on more discrete and non-handicapping signals such as, for example, body or urinary odors [21]. Most examples of mate preference for genetic compatibility are about assortative mating in hybrid zones and inbreeding avoidance

98 [22]. Both types of non-additive genetic effects may be achieved in various ways, including
99 mate choice based on genetic characteristics that would typically reveal kinship, for example
100 genes of the major histocompatibility complex (MHC) [23]. The MHC includes some of the
101 most polymorphic genes in vertebrates and plays a key role not only in immunity but also in
102 social communication [24]. The physiology of MHC-linked social signals is not sufficiently
103 worked out yet, but MHC genes have been found to influence social signaling in so many
104 different taxa that MHC-linked social signaling is likely the basis for chemical
105 communication in vertebrates [25]. If hybridization is to be avoided, somewhat similar MHC
106 genotypes would be preferred, as repeatedly observed at the level of gamete interaction [26-
107 29]. However, the role of the MHC in gamete fusion is not fully solved yet, as several studies
108 found no MHC effects at that level [30-32] while heterozygosity on the MHC sometimes
109 correlates with overall male fertility [33]. If inbreeding is to be avoided, similar MHC-
110 genotypes would generally be unattractive in mate selection [5,9,34], as seems to be the rule
111 in MHC-linked mate choice in other vertebrates, especially when tested on multiple MHC
112 loci [35,36].
113 Here we compare the potential benefits of preferring colorful males in good health
114 and vigor (i.e. 'good genes' sexual selection) to the potential benefits of preferring males
115 with complementary genotypes. We use the lake char (*Salvelinus umbla*) as model. This is a
116 non-migratory salmonid that is endemic to Alpine lakes and closely related to the Arctic char
117 (*S. alpinus*) [37]. While Arctic char often develop strong red breeding colorations in both
118 sexes [38], male lake char of our study population mostly develop a yellow breeding
119 coloration while females remain pale (Supplementary Fig. S1). In wild Arctic char,
120 coloration tends to increase with body length [39,40], and redder males have been observed
121 to show higher plasma testosterone levels [40], suffer less from infections [40], and show
122 lower lymphocyte counts [41] (but the link between coloration and stress indicators can be
123 broken up in captivity [38,42]). Wild lake char show similar patterns: more brightly colored
124 males are typically larger and suffer less from relative lymphocytosis and thrombocytosis,
125 two indicators of acute infections and hence of current health and vigor, than pale males [43].
126 Breeding colors of wild-caught char therefore fulfill key expectations for 'good-genes'
127 indicators in sexual selection [6]. We sampled breeders from the wild and used their gametes
128 in two types of breeding experiments, one that allows for inter-male sperm competition and
129 one that does not, to compare experimentally the genetic consequences of mating with a
130 brightly colored male versus mating with genetically dissimilar males that would result in
131 offspring of low inbreeding coefficients. We raised the resulting offspring under various

132 types of environmental stress to test whether and how male breeding colors or the genetic
133 similarity between males and females affect offspring stress tolerance.
134
135 **Results**
136 *Experiment 1*
137 The overall rate of embryonated eggs at 28 dpf (i.e. before treatments were applied) was
138 74.8% and varied between females (from 49.6% to 83.3%; $\chi^2 = 82.6$, df = 5, $p < 0.001$) and
139 males ($\chi^2 = 17.2$, df = 9, p = 0.046). This rate could not be predicted by male yellowness (r =
140 0.04, p = 0.90) but was significantly linked to the genetic distance between males and
141 females (kinship; r = 0.30, p = 0.04). Total late embryo mortality (from 28 dpf until and
142 including the day of hatching) was 3.0% and not linked to treatment ($\chi^2 = 0.39$, df = 2, p =
143 0.82).
144 There were strong family effects on embryo development rates as measured by
145 hatching date and hatchling length (see main family effects in Table 1). Mean hatching date
146 per family was also treatment dependent while hatchling size was not (family x treatment
147 interactions in Table 1), i.e. mean development rates per family varied with treatment. When
148 taking these family effects into account, neither hatching date nor hatchling length were
149 directly affected by the different treatments (main effects in Table 1).
150 The *a priori* prediction that embryo development would be positively linked to male
151 yellowness was not supported: Male yellowness did not predict the mean timing of hatching
152 and tended to be even negatively linked to hatchling (Fig. 1a,b), but this tendency was not
153 significant when tested directed (Table 1a; Figure 1a,b) and the significant interaction term
154 between treatment and yellowness (Table 1a) suggest that offspring of yellower males tend
155 to hatch later and of smaller size especially after exposure to *A. salmonicida*, i.e. they were
156 less tolerant to the pathogen stress than offspring of paler males. This was confirmed when
157 testing the effects of *A. salmonicida* and ethinylestradiol (EE2) separately: While EE2
158 showed no effects, exposure to *A. salmonicida* delayed hatching time (as expected, p_{dir} =
159 0.016; Table S1), and increasingly so with increasing male yellowness (tested directly
160 against expectation, p_{dir} = 0.024; Table S1).
161 The other *a priori* prediction, namely that embryo development rate would decline
162 with increasing genetic similarity between males and females (kinship), was supported here:
163 While hatching time was not significantly affected by kinship (Table 1b), hatchling size

declined with increasing kinship (Table 1b, Fig. 1c,d). These kinship effects on embryo development were similar in all treatment groups (Table 1b).

Experiment 2

Exposure to organic pollution reduced embryo development as it reduced hatchling length while not affecting hatching time and yolk sac volume (Fig. 2, Table 2). Fourteen days after hatching, larval length was still reduced by the treatment (Fig. S2, Table S2), while yolk sac volumes tended to be larger for the stressed larvae at that time than for the non-stressed ones (Table S2; Fig. S2).

As in the first experiment, there were again strong family effects on hatching time and hatchling length (Table 2, Table S2). While there were no significant general effects of treatment on hatching time, the stress treatment affected embryo development differently depending on family identity, i.e. the treatment differentially affected mean hatching time per family while hatchling length seemed unaffected (family x treatment interaction terms in Table 2).

Male yellowness did not significantly predict any offspring characteristics (main effects in Table 2a; Table S2a; Fig. 2, Fig. S2). However, the genetic distance between males and females (kinship) was a strong predictor of embryo and larval growth: With increasing kinship the larvae hatched at smaller size and with smaller yolk sacs despite hatching at similar times (Fig. 2d-f; Table 2b). Fourteen days after hatching, the effects of kinship on larval size were no more significant, but yolk sac volumes were still smaller with increased kinship (Fig. S2; Table S2).

Parental inbreeding coefficients

The inbreeding coefficients of the 10 males varied from -0.044 to 0.155 and showed higher variance than the inbreeding coefficients of the 5 females that could be genotyped (Levene's $F = 6.7$, $p = 0.02$; Fig. S3). The mean expected inbreeding coefficients of each parent's offspring (= mean kinship coefficients to mates) were positively correlated to the inbreeding coefficients of all 15 parents ($F = 13.3$, $n = 15$, $p = 0.003$; Fig. S3) or all 10 males ($F = 13.2$, $n = 10$, $p = 0.007$; Fig. S3). Nevertheless, male inbreeding coefficients did not negatively affect the rate of embryonated eggs in the first experiment ($r = 0.60$, $n = 10$, $p_{dir} = 0.14$) nor had any effects on offspring development in any of the experiments (Table S3).

Discussion

The strong family effects on embryo development that we observed here emphasize the need for controlling them in experimental studies. Treatment effect were generally low in both experiments because we deliberately kept them at sub-lethal level and hence induced rather benign stress. Nevertheless, effects of genetic similarity between parents were always large and in the expected direction, with higher similarity leading to reduced embryo development rates. Analogous large effects of genetic similarity could recently be demonstrated in an amphibian [44,45]. Male yellowness never predicted offspring performance in the direction that would be expected from 'good genes' hypotheses of sexual selection. This is in line with recent findings on three-spined stickleback (*Gasterosteus aculeatus*) where male coloration could not be linked to offspring viability [46].

Mating systems that are not based on resources, like those of salmonids, are a challenge to evolutionary biologists because female choice is expected to lead to increased offspring survival ('good genes') or increased attractiveness of sons [47]. A main challenge of the 'good genes' models is that females often prefer condition-dependent sexual ornaments that reveal current health and vigor and hence potentially the 'good genes' [e.g. 48,49], but it is not sufficiently solved yet what maintains the required additive genetic variance for fitness under such directed selection [50]. Various hypotheses have been suggested to possibly solve this 'lek paradox', including the idea that the condition dependence of the ornament reflects some key aspects of the interaction between a genome and a given environment [51,52]. Such genotype x environment interactions can affect the current fitness value of few important alleles, e.g. within the MHC where certain alleles provide protection against certain pathogens as observed, for example, in humans during the COVID19 pandemic [53], and as experimentally demonstrated in salmonids when exploring the within-family genetic variation for tolerance to bacterial infections [30,54]. In our first experiment, we used the same pathogen that Lohm et al. [54] used and could therefore assume significant genotype x environment interactions. It turned out that, contrary to predictions from 'good genes' models of sexual selection, male yellowness was negatively correlated to offspring development rate and pathogen resistance. We cannot fully exclude the possibility that we used a non-relevant pathogen in our first experiment and that yellowness would reveal genetic resistance against other types of pathogens. However, when we stimulated the growth of microbial pathogens that were already present on the eggs instead of adding new ones, male yellowness was still no useful predictor of offspring performance. Male yellowness was also no predictor of offspring tolerance against of the

common pollutant ethinylestradiol, the third stressor we used here at an ecologically relevant concentration that is known to be toxic for salmonids in early developmental stages [55-57].

Tomkins et al. [52] suggested that the 'good genes' hypothesis may be better described as 'absence of bad genes' because condition-dependent ornaments may reveal the absence of deleterious mutations. Indeed, mating success in seed beetles (*Callosobruchus maculatus*) could be experimentally linked to load of *de novo* mutations [58], but it remained unclear how much of this effect was due to female selection or male-male competition. Neff and Pitcher [59] demonstrated that classical non-additive genetic effects such as inbreeding can contribute to additive genetic variation for fitness because of genetic drift in finite populations. Such a heritability of inbreeding coefficients is expected to be highest in small or fragmented populations. Artificial selection experiments with *Drosophila* then revealed the expected link between male mating success and inbreeding depression: female mate preferences were consistent with avoidance of bad genes [60]. We found that parental inbreeding coefficients ($F_{\beta.parent}$) and even only male inbreeding coefficients ($F_{\beta.male}$) were correlated to the average kinship coefficients in our full-factorial breeding design and hence of the average expected inbreeding coefficients of their offspring ($\mu F_{\beta.offspring}$), i.e. we observed the correlation of inbreeding coefficients that is predicted for small populations such as the present one [61]. However, only $\mu F_{\beta.offspring}$ was a useful predictor of embryo performance, $F_{\beta.male}$ was not.

Variation in $F_{\beta.male}$ was also not linked to male yellowness [43], and we could not find any of the predicted positive links between male yellowness and male genetic quality as measured in their offspring performance. Male yellowness was, however, positively linked to male size and measures of milt quality and of general health and vigor as observed in a parallel study [43]. Male coloration in char may therefore first be an indicator of current fighting ability (or willingness to fight) and hence of dominance in male-male competition. However, we cannot exclude the possibility that male coloration reveals genetic variance for growth after the embryo stage [62].

In salmonids, the capacity of a male to dominate others seems to be an important predictor of male mating success in the wild [e.g. 63,64]. Our findings suggest that females get little genetic benefits from mating with such males. This supports analogous results from other salmonids [4]. It is possible that females benefit from the protection that dominant males sometimes provide against egg predation during the first minutes after spawning [65,66] or from higher fertilization success due to milt and sperm traits linked to male dominance [43,67]. However, if females aim for genetic benefits, we conclude from our

265 results that they prefer genetically dissimilar males with whom they would produce offspring
266 of low inbreeding coefficients. They could, for example, delay spawning, spawn only small
267 batches of eggs, and/or show false orgasms [8] when courted by a genetically more similar
268 but potentially dominant male. Petersson et al. [8] found that false female orgasms were less
269 likely with increasing number of males joining a dominant male for multi-male spawning,
270 and video recordings taken on natural spawning places revealed that female brown trout
271 sometimes prefer dissimilar phenotypes and thereby actively promote gene flow [68], but
272 this aspect of mate preference seems context dependent [69].
273 Salmonid fish are famous for their smelling ability in the context of migration and
274 homing [70]. They can also react strongly to odors of conspecifics as, for example, exposure
275 to female odors can quickly induce increased plasma testosterone levels in male Atlantic
276 salmon or brown trout [71]. Brown trout can recognize each other and profit from the
277 benefits that familiarity offers [72], and juvenile Arctic char learn to discriminate siblings by
278 their MHC and further still unidentified factors [73,74]. It is therefore possible that odors
279 reveal alleles, for example of the MHC via peptide ligands [75], that are used as markers for
280 the degree of kinship between males and females. MHC-linked mate preferences could then
281 be used to avoid genetically similar males, as has been found in a large number of other
282 vertebrates [25]. MHC effects on mate selection have indeed been discussed for various
283 salmonids [5,33,34,76-78].
284 It remains to be tested whether MHC-linked mate preferences can lead to inbreeding
285 avoidance in lake char and hence to the genetic benefits we observed. So far there seems to
286 be no other mechanism that would help to avoid inbreeding in salmonids [78]. One important
287 question for management is then whether mate preferences that help to avoid inbreeding, e.g.
288 MHC-linked mate preferences, can be innate in salmonids or have to be learned during
289 ontogeny as in other vertebrates [79,80]. Salmonid embryos show strong reactions to
290 chemical cues of their environment [81] and can be imprinted to certain odors at that stage
291 [82]. It is therefore possible that the expected high level of kinship among embryos who
292 develop as neighbors in the gravel of a natural spawning place allows for the development of
293 odor preferences that later help to avoid inbreeding during natural spawning. Most routine
294 procedures in hatcheries would then not only cancel potential effects of natural mate
295 preferences [83,84] or artificially boost the reproduction of sneaker males [85,86], but they
296 would even affect the development of adaptive mate preferences by raising embryos under
297 conditions that prevent the imprinting of adaptive odor preferences.
298

299 **Methods**

300 *Sampling and determining phenotypes and genotypes*

301 Wild lake char (10 males and 6 females) were caught in December 2017 from Lake Geneva
302 in gill nets, anaesthetized in 0.03% Eugenol (clove oil in ethanol at a 1:9 ratio),
303 photographed under standardized conditions (Fig. S1), and stripped for the gametes. These
304 gametes were then used for 2 different fertilization experiments as explained below.

305 Male breeding coloration (that is mostly yellow in the study population) was
306 analyzed as described in Nusbaumer et al. [43]. Briefly, yellowness was measured from the
307 photos as the *a** and *b** components of the CIE-*L*a*b** color space, respectively (the a* axis
308 for greenness-redness and the b* axis for blueness-yellowness both range from -100 to 100).
309 The means of three squares in the pectoral region, the ventral region and the anal region were
310 used as measures of male yellowness as in Parolini et al. [87].

311 DNA was extracted from the samples and processed as described in Nusbaumer et al.
312 [43] for the 14 parents that were used on both experiments. Briefly, the enzymes EcoRI-HF
313 and MspI (New England Biolab, Ipswich MA, USA) were used for DNA digestion and a
314 unique EcoRI barcode was ligated to each individual, following the protocol as Brelsford et
315 al. (2016). After PCR, fragments were single-end genotyped on 2 lanes of Illumina HiSeq
316 2500 with fragments of 125 bp length at Lausanne Genomic Technologies Facility
317 (University of Lausanne). The quality of the fastq files was assessed using fastqc v 0.11.4
318 [88]. Despite the phred quality score was higher than 30 until base 114, read were trimmed to
319 110 bp using trimmomatic [89] and mapped to the *Salvelinus* spp. reference genome [90]
320 using BWA [91]. BAM files were analyzed in Stacks 1.48 [92]. Using vcftools [93], loci
321 were filtered for a coverage of between 10X and 50X, presence in all individuals, and for
322 Hardy-Weinberg equilibrium. This led to a total of 4,150 SNPs with a mean coverage of
323 29X. Individual inbreeding coefficients ($F_{\beta.parent}$) and kinship ($\mu F_{\beta.offspring}$) were estimated
324 using the *beta.dosage* function of the package *Hierfstat* [94].

325 The two further females that were only used in the first experiment were processed in
326 a separate library using the same enzymes and the PCR settings as for the second
327 experiment. The size selection has been made using the Blue Pippin Cassette 2% with
328 internal marker V1 (Sage Science, Massachusetts, USA) selecting fragments from 400 to 600
329 bp. The resulting library was sequenced on one lane of Illumina HiSeq 2500 with a fragment
330 size of 150 bp. The quality of the fastq files was assessed using fastqc v 0.11.4 [88]. Read
331 were again trimmed to 110 bp to analyze these individuals with those genotyped on the
332 previous library. All BAM files of the 16 parents were processed using stacks 2.53 [95]. In

333 the VCF obtained using populations only SNPs present in at least 80% of individuals (-*r* 0.8)
334 and with a maximum observed heterozygosity of 0.5 per locus (--*max-obs-het* 0.5) were
335 retained. Using vcftools [93] SNPs were retained only if having a minimum coverage of 10X
336 (--*minDP* 10), a maximum coverage of 50X (--*maxDP* 50), presence in all the individuals (--
337 *max-missing* 1) and at Hardy-Weinberg equilibrium (--*hwe* 0.05). One of the two additional
338 females was excluded from the analysis because of the low genotyping rate and low
339 coverage (more than 60% of missing data; mean coverage 12X). This led to a final VCF with
340 a total of 2,199 SNPs with a mean coverage of 26.3X
341 All males and 5 of the 6 females could be genotyped, i.e. all 60 full-sib families could
342 be used to test for effects of male yellowness or $F_{\beta.male}$ on embryo performance after they had
343 hatched (experiment 1, see below) while 50 and 40 families could be used to test for effects
344 of kinship on embryos in experiment 1 and 2, respectively.
345
346 *Experiment 1*
347 Milt was stripped from the males into individual 50 mL Falcon tubes (Greiner Bio-One,
348 Austria), carefully avoiding contamination by water, urine or blood, then mixed 1:9 with
349 diluted Storefish© (IMV Technologies, France; diluted 1:9 with MilliQ water) and stored on
350 ice. The next day, eggs were stripped from the females into individual plastic containers and
351 then about equally distributed to 20 Petri dishes (Greiner Bio-One, Austria) per female. Only
352 10 batches per females were used here, the other batches were fertilized with milt from males
353 from a captive breeding stock in the context of another study on genetic management of
354 captive stocks (Garaud et al., unpublished). Diluted milt was then added to the individual
355 batches of eggs before standardized water [96] was added to activate the sperm and induce
356 fertilizations to produce all possible 60 families (6 females x 10 males). After letting the
357 freshly fertilized eggs harden for 2 hours, they were transported on ice to a climate chamber
358 where they were washed and singly distributed to 24-well plates (each embryo in its own
359 compartment filled with 1.8 mL autoclaved standardized water) and raised at 4.5°C
360 following the protocol developed by von Siebenthal et al. [97]. The rate of embryonated eggs
361 was determined at 28 dpf (days past fertilization). Late embryo mortality and day of hatching
362 was then determined for the remaining 1,073 visible embryos.
363 On day 55 after fertilization, the embryos were exposed to one of three treatments by
364 adding 200µL to the well with either (i) *Aeromonas salmonicida* (10^6 cfu), (ii) 2 pg
365 ethinylestradiol ("EE2"), or (iii) 100µL of standardized water plus 100µL of MilliQ water

366 (sham exposed). For the first treatment, dry-frozen *A. salmonicida* that had previously been
367 isolated from lake char were rehydrated and inoculated at 22°C for 24 hours in TBS (Tryptic
368 Soy Broth, Fluka, Switzerland). Cultures were then washed and counted using a Helber
369 counting chamber as in Clark et al. (2013). Bacteria were diluted in autoclaved standardized
370 water and 1% TSB such that adding 100µL in each well plus 100µL of MilliQ water would
371 result in 0.5 x 10^4 cfu per mL water in the well. For the second treatment, an EE2 stock
372 solution was prepared as in Marques da Cunha et al. [56]. Briefly, analytical 17alpha-
373 ethinylestradiol (Sigma-Aldrich, USA) was diluted in MilliQ water and 0.004% absolute
374 ethanol (VWR International, USA) to a stock solution of 20 ng EE2/L. The 100µL of this
375 solution plus the 100µL of standardized water that were then added to the wells resulted in
376 an initial concentration of 1 ng/L. These 2pg EE2/well can be expected to be largely
377 absorbed by the embryo within the following 4 weeks [56]. Embryo and larval mortality
378 were monitored from then on. At the day of hatching, in total 1,065 larvae were transferred
379 to 5mL wells with standardized water. Standard photos were taken on the same day to
380 determine larval length in ImageJ.
381
382 *Experiment 2*
383 After diluting the milt of each male in the Storfish solution (see above), sperm motility was
384 determined to prepare standard dilutions of 25 million active sperm/mL [details in 43]. The
385 standard dilutions of 2 males each (haphazardly assigned from the 10 males) were then used
386 to fertilize in total 80 batches of 24 eggs each of 4 of the 6 females that had been used in
387 experiment 1 to test for potential effects of ovarian fluids on sperm competition in a fully-
388 factorial experimental setup [43]. Ten of these 80 experimental cells were accidentally lost
389 (all of the same female exposed to sperm activated in water only), reducing the number of
390 eggs to be monitored to N = 1,680. These eggs were batch-wise shortly rinsed in cold tap
391 water for 30 s (4 L/min) and distributed singly to 24 well plates. Because the sperm
392 competition trials were done with or without the presence of ovarian fluids and the rinsing of
393 the eggs was done such that remainders of ovarian fluids could be expected to support
394 microbial communities of the developing eggs as in Wedekind et al. [98]. Apart from this
395 organic pollution, no other stress treatment was applied. Embryos were monitored as in
396 experiment 1, with additional measurements of yolk sac volumes at the day of hatching and
397 14 days later when larvae were euthanized and genotyped for 3 to 6 microsatellite markers to
398 identify their fathers [details in 43]. Yolk sac volume was determined as in Jensen et al. [99].

All embryos and larvae that had died before were also genotyped. A sex-specific marker was added to the first multiplex of 3 microsatellite markers to test for sex-specific effects on embryo development that are reported in Nusbaumer et al. [100].

Statistics

Statistical analyses were done in JMP 15.2.1. When using linear mixed models, family identity and their interactions with treatments were included as random factors while treatment, male yellowness or genetic distance between males and females (kinship) were used as fixed factors. Directed tests were used based on the *a priori* predictions that (i) if male yellowness is a good-genes indicator, embryo development is expected to be positively linked to their fathers' yellowness, and (ii) if the kinship between the parents and hence the expected mean inbreeding coefficient of their offspring affects their development, kinship is expected to the negatively correlated to embryo development rate. Directed tests were used because such tests avoid inflation of the alpha value while still allowing to discover effects in the non-expected direction if the effect sizes are strong [101]. The corresponding p-values are notated as p_{dir}.

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Table 1. First experiment: Linear mixed model on embryo hatching time (dpf) and hatchling length when predicted by treatment and (a) male skin coloration (yellowness), (b) the genetic similarity between males and females (kinship). Hatching date was included as further fixed factor when testing for effects on hatchling size. Family identity and the interaction between family ID and treatment were included as random factors. Main effects were tested directed (p_{dir}) based on the *a priori* predictions that male yellowness would be positively, and kinship negatively, linked to embryo development. Significant p-values are highlighted in bold.

		Hatching time			Hatchling size			
Effects	d.f.	F	Variance component	p	F	Variance component	p	p_{dir}
(a) Male skin coloration								
Treatment	2	2.7		0.07	0.3		0.73	
Yellowness	1	0.1		0.72	4.7		0.036	0.07
Treatment x yellowness	2	3.3		**0.04**	1.6		0.21	
Hatching date	1				0.1		0.75	
Family ID[1]			1.93±0.42	**<0.001**		0.02±0.007	**0.002**	
Family x treatment[1]			0.51±0.14	**0.001**		<0.001±0.005	0.87	
Residual			3.16±0.15			0.23±0.01		
(b) Male & female kinship								
Treatment	2	1.4		0.25	1.7		0.58	
Kinship	1	1.2		0.27	8.2		0.06	**0.04**
Treatment x kinship	2	0.2		0.84	1.3		0.55	
Hatching date	1				12.8		0.19	
Family ID[1]			1.99±0.4**8**	**<0.001**		0.03±0.009	**0.004**	
Family x treatment[1]			0.46±0.15	**0.002**		0.002±0.006	0.79	
Residual			3.40±0.18			0.22±0.01		

[1]random effects, REML unbounded variance components ± standard error, Wald p-values

Table 2. Second experiment: Linear mixed model (REML, unbounded variance components) on embryo hatching time (dpf) and hatchling length when predicted by treatment and (a) male skin coloration (yellowness) or (b) the genetic similarity between males and females (kinship). Hatching date was included as further fixed factor when testing for effects on hatchling size. Family identity and the interaction between family ID and treatment were included as random factors. Significant p-values are highlighted in bold.

		Hatching time			Hatchling size			Yolk sac volume		
Effects	d.f.	F	Variance component	p	F	Variance component	p	F	Variance component	P
(a) Male skin coloration										
Treatment	1	0.3		0.56	19.0		**<0.001**	0.1		0.74
Yellowness	1	0.4		0.53	0.9		0.36	0.01		0.93
Treatment x yellowness	1	0.4		0.54	0.4		0.54	0.02		0.90
Hatching date	1				36.6		**<0.001**	7.8		**0.005**
Family ID[1]			0.70±0.20	**<0.001**		0.05±0.01	**<0.001**		10.4±2.8	**<0.001**
Family x treatment[1]			0.12±0.06	**0.04**		0.004±0.004	0.26		0.5±0.6	0.42
Residual			2.18±0.09			0.16±0.01			35.0±1.4	
(b) Genetic similarity										
Treatment	1	0.3		0.59	20.9		**<0.001**	0.02		0.89
Kinship	1	0.003		0.96	7.6		**0.009**	15.0		**<0.001**
Treatment x kinship	1	0.02		0.90	3.0		0.09	0.04		0.84
Hatching date	1				35.5		**<0.001**	6.7		**0.01**
Family ID[1]			0.70±0.20	**<0.001**		0.04±0.01	**<0.001**		6.9±2.0	**<0.001**
Family x treatment[1]			0.12±0.06	**0.04**		0.003±0.003	0.42		0.5±0.7	0.47
Residual			2.18±0.09			0.16±0.01			35.0±1.4	

[1]random effects, REML unbounded variance components ± standard error, Wald p-values

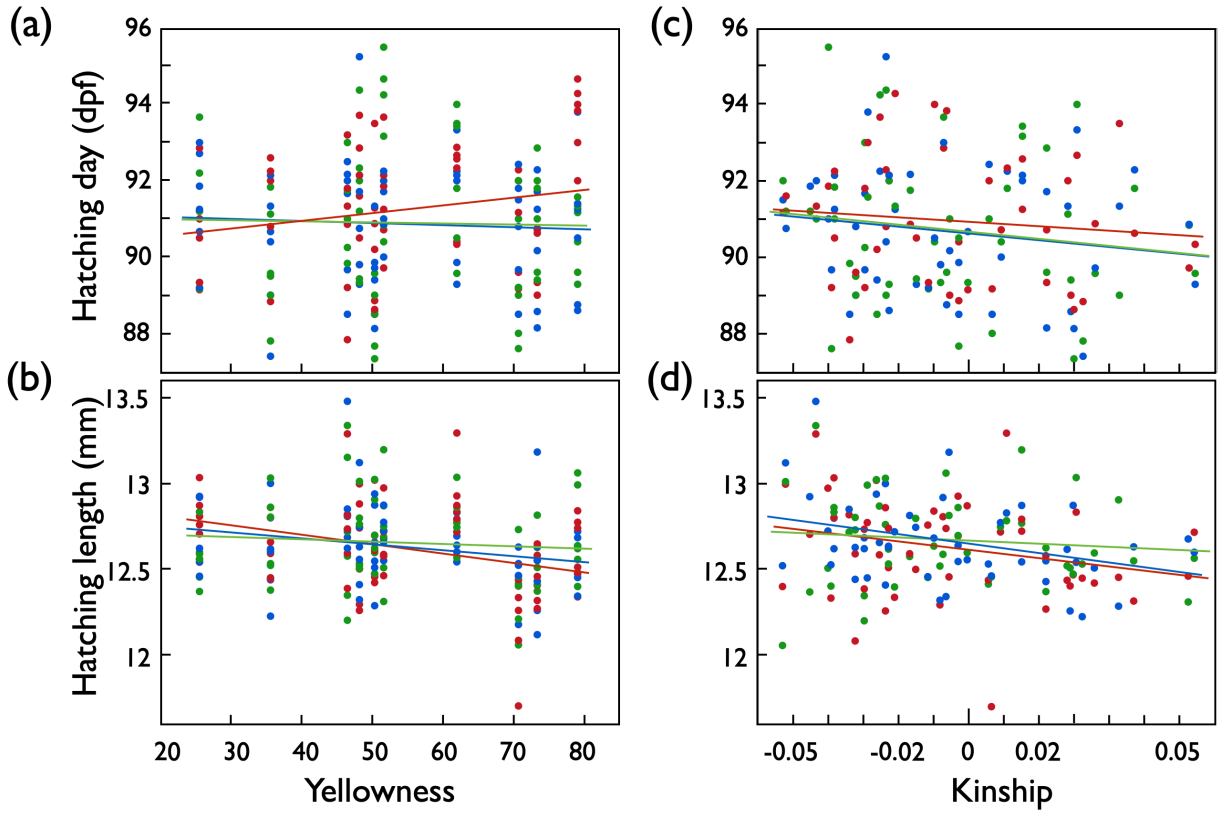

Figure 1. First experiment: Embryo development after exposure to *Aeromonas salmonicida* (red symbols and regression lines), 1 ng/L ethinylestradiol (green), or sham exposure (blue). (a,b) Mean hatching time (dpf == days past fertilization) and mean hatchling length per full-sib family predicted by male skin coloration (yellowness), and (c,d) predicted by the genetic similarity between males and females (kinship). See Table 1 for statistics.

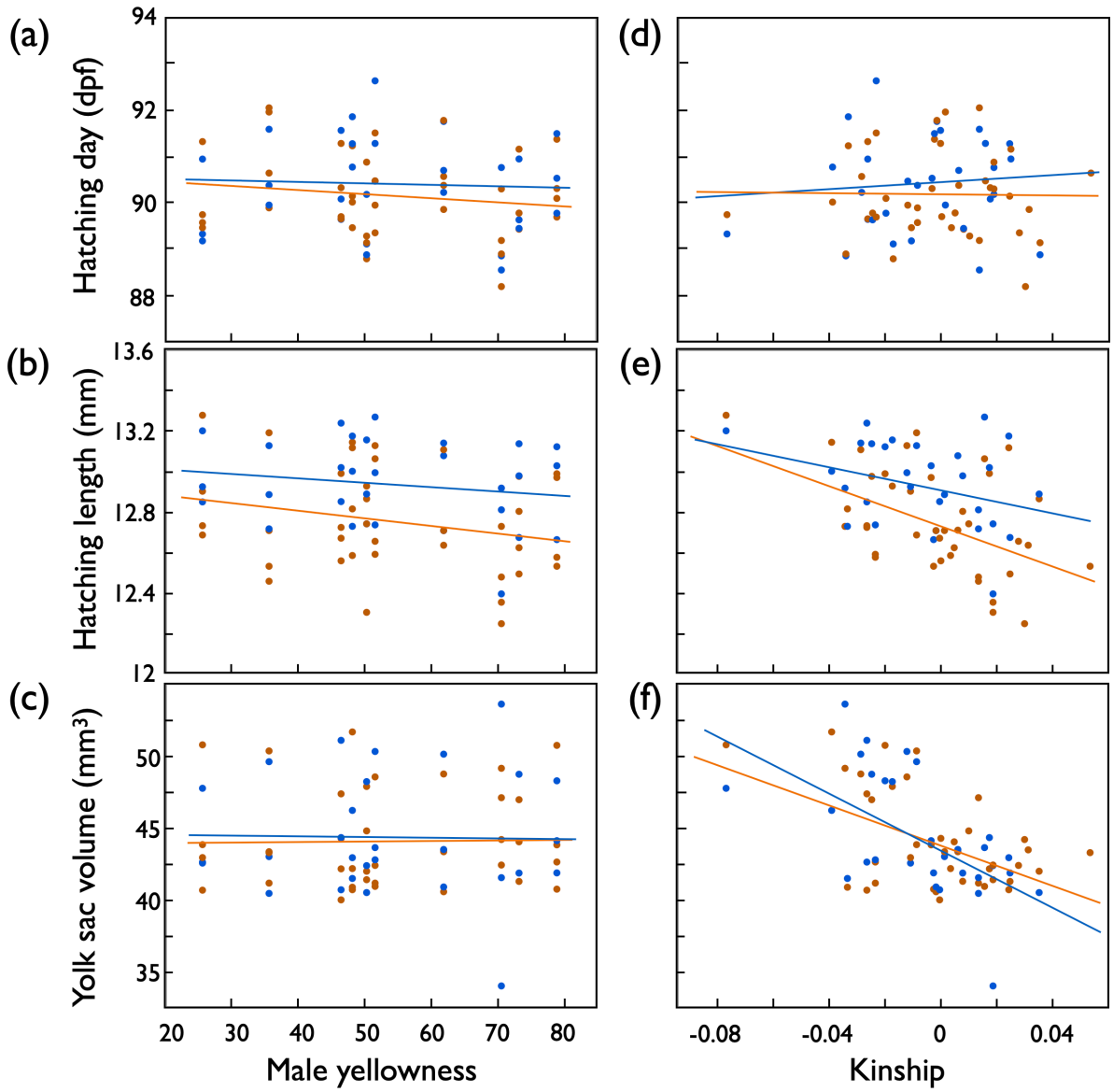

Fig. 2. Second experiment (fertilisation during sperm competition): hatching time (dpf, days past fertilization), hatchling length, and yolk sac volume of hatchlings (means per full-sib family) predicted by (a,b,c) male skin coloration (yellowness), (d,e,f) the genetic similarity between males and females (kinship) after exposure to organic pollution (orange symbols and regression lines), or not exposed (blue). See Table 2 for statistics.

Supplementary information

Genomic compatibility excels possible 'good genes' effects of sexual selection in lake char

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Content

Table S1. Parameter estimates for the linear mixed model presented in Table 1a (1st experiment).
Table S2. Testing for effects on larval growth in 2nd experiment
Table S3. Testing for effects of male inbreeding coefficients

Figure S1. Length-standardized photos of a male and a female lake char during spawning season.
Figure S2. Sampling locations
Figure S3. Larval length 14dph predicted by treatment and male characteristics

Supplementary Table S1: Parameter estimates for the linear mixed model presented in Table 1a (1st experiment). Effects on embryo hatching time and hatchling length when predicted by the treatments *A. salmonicida* and ethinylestradiol ("EE2") and male skin coloration (yellowness). Directed p-values (p_{dir}) were calculated if two-tailed p-values were < 0.1 based on the *a priori* predictions that male yellowness could be an indicator of good genes and would then be positively linked to embryo development. Significant p-values are highlighted in bold.

	Estimate ± SE	t	p	p_{dir}
(a) Hatching time				
Intercept	90.73±0.69	132.2	**<0.0001**	
A. salmonicida	0.25±0.11	2.3	0.025	**0.016**
EE2	-0.08±0.11	-0.8	0.44	
Male yellowness	0.004±0.01	0.4	0.72	
A. salmonicida x yellowness	0.02±0.007	2.6	0.012	**0.024**
EE2 x yellowness	-0.008±0.007	-1.2	0.25	
(b) Hatchling length				
Intercept	12.60±0.65	19.3	**<0.0001**	
A. salmonicida	-0.01±0.02	-0.5	0.62	
EE2	0.02±0.02	0.8	0.44	
Male yellowness	-0.003±0.001	-2.2	0.036	0.072
A. salmonicida x yellowness	-0.002±0.001	-1.3	0.18	
EE2 x yellowness	0.002±0.001	1.7	0.10	
Hatching time	0.001±0.007	0.3	0.75	

Supplementary Table S2. Testing for effects on larval growth in 2nd experiment: Linear mixed model on larval length 14 days post hatching (dph) when predicted by treatment and (a) male skin coloration (yellowness) or (b) the genetic similarity between males and females (kinship). Hatching date was included as further fixed factor. Family identity and the interaction between family ID and treatment were included as random factors. Significant p-values are highlighted in bold.

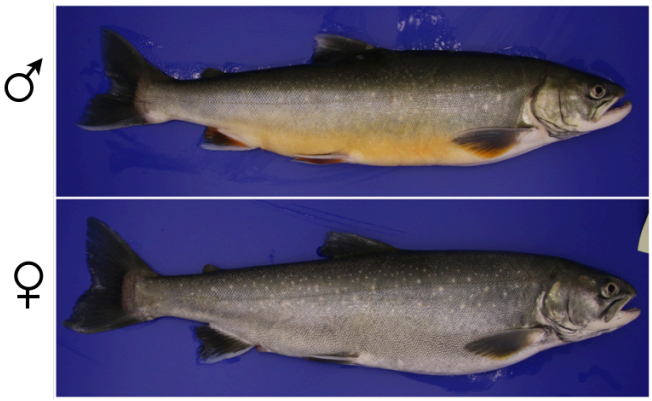
		Larval size 14 dph			Yolk sac volume 14 dph		
Effects	d.f.	F	Variance component	p	F	Variance componen t	p
(a) Male skin coloration							
Treatment	1	17.1		**<0.001**	3.4		0.08
Yellowness	1	0.9		0.95	0.2		0.65
Treatment x yellowness	1	0.9		0.88	0.9		0.34
Hatching date	1	38.0		**<0.001**	79.5		**<0.001**
Family ID[1]			0.13±0.04	**0.001**		2.01±0.76	**0.008**
Family x treatment[1]			0.05±0.02	**0.02**		0.28±0.44	0.52
Residual			0.65±0.03			26.8±1.3	
(b) Male & female kinship							
Treatment	1	17.4		**<0.001**	4.5		**0.04**
Kinship	1	2.9		0.09	14.3		**<0.001**
Treatment x kinship	1	1.2		0.29	0.7		0.40
Hatching date	1	37.3		**<0.001**	86.9		**<0.001**
Family ID[1]			0.12±0.04	**0.002**		1.13±0.59	**0.008**
Family x treatment[1]			0.04±0.02	**0.03**		0.25±0.46	0.52
Residual			0.65±0.03			26.8±1.1	

[1]random effects, REML unbounded variance components ± standard error, Wald p-values

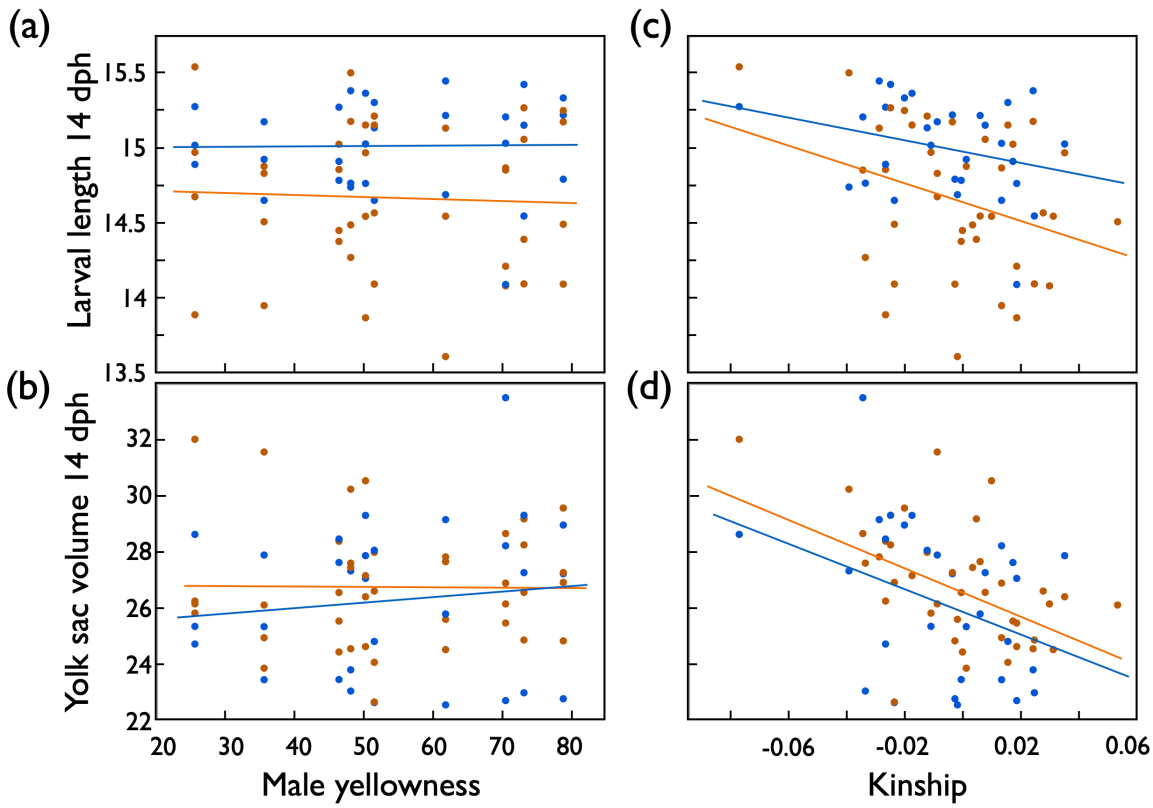
Supplementary Table S3. Testing for effects of male inbreeding coefficients: Linear mixed model on embryo hatching time (dpf), hatchling length, and yolk sac volume at hatching when predicted by treatment and male inbreeding coefficient ($F_{\beta.male}$) in (a) the first, and (b) the second experiment. Hatching date was included as further fixed factor when testing for effects on hatchling size. Family identity and the interaction between family ID and treatment were included as random factors. P-values are highlighted in bold if significant for fixed factors.

		Hatching time			Hatchling size			Yolk sac volume		
Effects	d.f.	F	Variance component	P	F	Variance component	P	F	Variance component	P
(a) First experiment										
Treatment	2	2.5		0.09	0.3		0.73			
$F_{\beta.male}$	1	<0.1		0.93	0.5		0.82			
Treatment x $F_{\beta.male}$	2	1.1		0.32	0.9		0.66			
Hatching date	1				0.2		0.93			
Family ID[1]			1.93 ±0.43	**<0.001**		0.03±0.008	**0.001**			
Family x treatment[1]			0.55±0.14	**<0.001**		<0.001±0.005	0.97			
Residual			3.16±0.15			0.21±0.01				
(b) Second experiment										
Treatment	1	0.3		0.56	19.1		**<0.001**	0.1		0.77
$F_{\beta.male}$	1	0.1		0.71	0.02		0.90	0.01		0.93
Treatment x $F_{\beta.male}$	1	0.1		0.73	1.1		0.31	1.1		0.31
Hatching date	1				37.2		**<0.001**	7.9		**0.005**
Family ID[1]			0.70±0.20	**<0.001**		0.05±0.01	**<0.001**		10.4±2.8	**<0.001**
Family x treatment[1]			0.12±0.06	**0.04**		0.004±0.004	0.33		0.4±0.6	0.49
Residual			2.18±0.09			0.16±0.01			35.0±1.4	

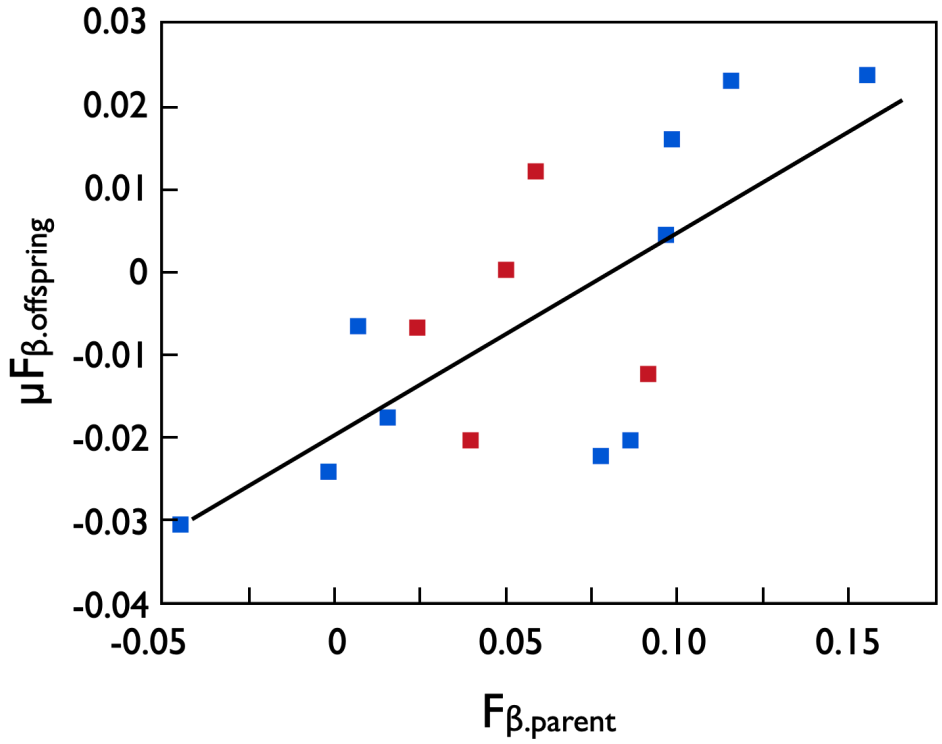
[1]random effects, REML unbounded variance components ± standard error, Wald p-values



Supplementary Figure S1. Size-standardized photos of a male (about median yellowness) and a female lake char (*Salvelinus umbla*).



Supplementary Figure S2. Larval growth in 2nd experiment 2: Mean larval length (mm) 14 days past hatching (dph), and mean yolk sac volume at that time (means per full-sib family) predicted by (a,b) male skin coloration (yellowness) or (c,d) by the genetic similarity between males and females (kinship) after exposure to organic pollution (orange symbols and regression lines), or sham exposed (blue). See Table S1 for statistics.



Supplementary Figure S3. The mean expected inbreeding coefficients of each parent's offspring ($\mu F_{\beta.offspring}$, corresponds to the mean kinship coefficients to mates in the full-factorial breeding design) predicted by the parental inbreeding coefficients ($F_{\beta.parent}$; females red, males blue). The line gives the regression. See text for statistics.